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## GLUCOCORTICOIDS AND EXPERIMENTAL PRODUCTION OF GASTRIC ULCERATIONS

BY

**T. O. KEYRILÄINEN, R. VASAMA, M. ISOMÄKI and J. L. KALLIOMÄKI**

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# GLUCOCORTICOIDS AND EXPERIMENTAL PRODUCTION OF GASTRIC ULCERATIONS

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*T. O. KEYRILÄINEN, R. VASAMA, M. ISOMÄKI and J. L. KALLIOMÄKI*

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## INTRODUCTION

There are numerous clinical reports suggesting that during cortisone, hydrocortisone, and prednisone therapy there might occur dyseptic symptoms, gastrointestinal haemorrhages, activations of cured peptic ulcers, fresh ulcers, and peptic ulcer perforations, even fatal ones (2, 23, 25, 35, 8, 28, 3, 6, 16, 27, 29, 5, 12, 13, 19, 31, 4, 10, 18, 20, 21, 37). Most of these complications seem to be caused prednisone. Boland (5) mentions that when hydrocortisone therapy was changed into prednisone therapy, the frequency of the ulcers increased from 5.5 to 31 %. Dyspepsia that occurred during prednisone therapy seemed to disappear when they gave up prednisone for hydrocortisone treatment (16). In a series of cases treated with prednisone, peptic ulcer occurred in 16 cases, usually appearing during the 3rd or 4th week after the beginning of treatment (4). Kern *et al.* (21) think that the risk of ulcers during corticosteroid therapy rises 20 — 60-fold if the maintenance dose of cortisone is above 50 mg/day and that of prednisone more than 20 mg/day. If the maintenance dose is below these figures, the risk of ulcers scarcely differs from normal. Henderson (17) found an incidence of 5.3 % of ulceration in reviewing a series of 1400 patients treated with cortisone. At the same time the ulcer incidence in general adult population was calculated to be 6 %, suggesting that cortisone treatment had not increased the incidence of ulcer.

The mechanism causing the gastrointestinal complications during corticosteroid therapy is still unknown. We know that the corticosteroids, like ACTH, stress, and Cushing's syndrome, lead to increased gastric secretion in man (38, 14, 34, 15, 24, 1, 7). The increased gastric secretion caused by corticosteroids and stress can be prevented by anticholinergic drugs and, according to same authorities, by the gastrointestinal complications induced by corticosteroid therapy (34, 11, 7). The increased gastric secretion induced by corticosteroids is independent of vagal

stimulation (38). Daily doses of 10 mg of prednisone or 40 mg of hydrocortisone do not influence the secretion of uropepsin; if on the other hand, these doses are doubled, the secretion of uropepsin increases noticeably (30). Carbone *et al.* (7) write: "apparently prednisone enhances the effect of acetylcholine either by sensitizing the parietal cell to its action or, more directly, by increasing its production or decreasing its destruction".

The influence of corticosteroids upon gastric ulcerations induced in testanimals by various methods has also been studied. Crane and Duncan (9) found that intramuscularly-given cortisone increased and deepened the ulcreations caused in a rabbit's stomach by pitressin injections. Further, they observed that an invasion of the stomach by bacteria, in particular by Cl. welchii and coliform bacilli, was increased and that perforations resulted in a few cases during cortisone administration.

Rodriquez-Olleros and Galindo (33) studied the influence of cortisone and ACTH upon ulcers and gastroduodenitis induced in dogs by Atophan. By administering these drugs it was possible to retard the healing process of duodenal lesions to some extent, but they had no influence upon the healing tendency of the gastric lesions. In Atophan-induced ulcers cortisone and ACTH did not give the maximum response to Atophan and did not prevent the healing of ulcers when the administration of Atophan was stopped.

When investigating into the influence of certain hormones on pylorus-ligated rats, van Geertryden *et al.* (36) noticed that both the androgens and the estrogens, DOCA and ACTH slightly decreased gastric secretion and the HCl content of the gastric juice, while cortisone increased both the rate of the secretion and the HCl concentration. None of these hormones seemed to influence the extent of peptic ulcreations. Remouchamps and Merck (32) believe, however, that in Shay rats given ACTH or cortisone gastric ulcers perforate more often than in untreated controls. While administering 0.5 mg/kg of prednisone or cortisone orally to rats for 11 days before pylorus ligation, Mainardi (26) did not observe any differences in ulceration from controls. An increase of the dose to 5 mg/kg enhanced the formation of ulcers, most clearly in those rats

which had received cortisone. Mainardi fed and gave parenterally cortisone and prednisone doses of various size to healthy rats, but he could not observe any changes in the mucous membranes of their stomachs even after a treatment lasting 60 days.

In the study now reported we tried to elucidate in what degree hydrocortisone and prednisone influence locally the mucous membrane of the stomach. If ulceration due to cortisone preparations is chiefly due to the immediate effect of these preparations upon tissues and cells, it seemed reasonable to expect that by applying cortisone preparations in different ways to the stomach wall some light could be shed on the mechanism of that effect.

## MATERIAL AND METHODS

The series consisted of 63 male albino rats weighing about 170 — 255 g. Upon 31 of the rats various types of hydrocortisone and prednisone applications were made through the serosa into the stomach wall. Upon 32 rats a pylorus ligation was performed, in order to study the influence of prednisone on the formation of ulcers and the secretion conditions in the stomach.

Before the application of hydrocortisone and prednisone into the stomach wall the rats were anaesthetized with ether. Through an incision at the midline the stomach was pulled out and in the serosa at the anterior wall of the stomach of 10 rats an incision about 3 mm long was made, through which subserosally an approximately 1 mm thick piece of a hydrocortisone tabloid (Korti tabl. Lääke Oy [Ltd.] containing hydrocortisone as alcohol) was bluntly pushed with thin tweezers about 1.5 cm. The hydrocortisone dose was then about 1 mg. The application of the tabloid piece succeeded easily without haemorrhage. The incision on the serosa was not closed, the stomach being pushed back into the abdomen cavity and the incision closed with silk sutures. After operation the rats were allowed to live and eat freely; they were killed 3 — 14 days later. Instead of the hydrocortisone tabloid piece 10 rats received an injection of hydrocortisone acetate suspension (Korti susp., Lääke Oy, Ltd.), likewise into the stomach wall. The dose of hydrocortisone acetate

was about 1 mg; it was injected, together with an antibiotic suspension containing about 40000 I.U. of penicillin and 0.1 g of streptomycin. The rats were killed 1 — 4 days after the operation. Instead of hydrocortisone acetate a water suspension made of a prednisone tabloid in such a way that the prednisone dose was 0.7 mg was injected into 8 rats. The animals were also given the same amount of antibiotics, but this time straight into the abdominal cavity and no longer into the stomach wall. With 4 rats the same procedure was performed as when the hydrocortisone tabloid was put into the stomach wall, but this time the piece did not contain hydrocortisone at all, only the other ingredients of the tabloid. No antibiotics were administered to these rats either. They were killed 1 — 3 after the operation. Microscopic examination was carried out with van Gieson stained sections fixed in 4 % neutral formaldehyde.

Pyloric ligation was made on 32 rats by the technique described in detail earlier (22). Before the ligation, the rats had been fasted for 60 hours; the pylorus was ligated 21 hours before the animals were killed. Immediately after the ligation, 10 rats received, by means of a catheter, 1 mg of prednisone tabloid suspended in water into the stomach. The tabloids did not contain alkali. 10 rats were administered the same dose of prednisone injected straight into the small intestine; accordingly, it did not affect the stomach at all, but produced a systemic effect of the prednisone. 12 rats functioned as controls and were treated in the same way, but were not given any prednisone at all. When the animals were killed, the examinations of the gastric juice and the evaluation of the ulceration were made in the way described in the above-mentioned report (22).

## RESULTS

The results are presented in tables 1 and 2.

## EXPLANATIONS OF PLATES

*Plate 1.* A section of the stomach wall 3 days after the application of a hydrocortisone tabloid. No antibiotic treatment. A typical nearly perforated ulcer with the edge undermined. The surrounding submucosa and the muscularis propria are severly inflammated. (van Gieson; 30 x.)

*Plate 2.* A section of the stomach wall 4 days after application of hydrocortisone and antibiotic suspension. The suspension is in the submucosa in which small hemorrhages and signs of slight inflammation are to be seen. The mucosa is intact. (van Gieson; 30 x.)

*Plate 3.* As in Fig. 2, but after 2 days of application. The suspension is in the submucosa between the muscularis mucosae and the muscularis propria. In the suspension there are some macrophages, leukocytes an erythrocytes. The mucosa is intact. (van Gieson; 120 x.)

*Plate 4.* Four days after the application of prednisone suspension into the stomach wall and antibiotic suspension intraperitoneally. The suspension is in the submucosa where connective tissue formation and slight inflammatory infiltration are to be seen around the place of application. The mucosa is intact. (van Gieson; 30 x.)

*Plate 1*

*Plate 2*

*Plate 3*

*Plate 4*

Table 1

The results of different types of application of hydrocortisone and prednisone into the stomach wall.

| Type of Procedure | Time of Influence (Days) | Microscopic Findings | | | Macroscopic Findings | | |
|---|---|---|---|---|---|---|---|
| | | Ulcer | Inflammation | Haemorrhages | Ulcer | Inflammation | Haemorrhages |
| A piece of hydrocortisone tablet between serosa and muscularis mucosae .... | 3 | + | + | + | + | + | — |
| — „ — | 3 | + | + | — | + | + | — |
| — „ — | 3 | + | + | — | + | + | — |
| — „ — | 4 | + | — | — | + | + | — |
| — „ — | 4 | + | — | — | + | + | + |
| — „ — | 4 | — | — | — | + | + | — |
| — „ — | 9 | + | + | — | — | + | — |
| — „ — | 9 | +(?) | + | — | + | + | — |
| — „ — | 14 | + | — | — | — | — | — |
| — „ — | 14 | + | + | — | — | + | — |
| Suspension of hydrocortisone acetate and penicillin + streptomycin between serosa and muscularis mucosae ..... | 1 | — | — | + | + | + | + |
| — „ — | 1 | — | — | + | + | — | + |
| — „ — | 1 | — | — | + | — | — | + |
| — „ — | 1 | — | — | + | — | — | + |
| — „ — | 2 | — | — | + | — | — | + |
| — „ — | 2 | +(?) | — | — | + | + | + |
| — „ — | 2 | — | — | — | — | — | — |
| — „ — | 4 | +(?) | — | + | — | — | — |
| — „ — | 4 | — | — | + | + | + | + |
| Suspension of prednisone between serosa and muscularis mucosae, penicillin + streptomycin intraperitoneally ..... | 2 | — | — | + | — | — | — |
| — „ — | 2 | — | — | + | — | + | + |
| — „ — | 4 | — | — | + | — | + | — |
| — „ — | 4 | — | — | — | — | + | — |
| — „ — | 6 | + | — | — | — | + | — |
| — „ — | 6 | — | — | — | — | + | — |
| — „ — | 12 | — | — | — | — | + | — |
| — „ — | 12 | + | — | — | + | + | + |
| A piece of placebo tablet between serosa and muscularis mucosae .... | 1 | +(?) | + | — | — | + | + |
| — „ — | 1 | — | + | + | + | + | + |
| — „ — | 3 | — | + | — | + | + | + |
| — „ — | 3 | + | — | + | — | + | + |

Table 2

The influence of prednisone administration on pylorus ligated rats, the drug being was injected into the stomach and the intestine.

| Group | No. of Rats | Stomach Wall | | | | | | | Gastric Juice | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of Ulcers Grouped according to Diameter of Ulcer | | | | No. of Perforations | No. of Unaffected Stomachs | "Ulceration Index" | Secretion ml/100 g/hr $\bar{x}$ (R) | pH $\bar{x}$ (R) | Free HCl / 100 ml $\bar{x}$ (R) | Total Acidity / 100 ml $\bar{x}$ (R) | Peptic Activity in Mett Units $\bar{x}$ (R) | Relative Peptic Conc. in Mett Units $\bar{x}$ (R) |
| | | 0—2 mm | 2—5 mm | 5—10 mm | >10 mm | | | | | | | | | |
| **Prednisone in Stomach 1 mg after Ligation of Pylorus** | 10 | 32 | 7 | 1 | 1 | 0 | 3/10 | 9.4 | 0.38 (0.47—0.31) | 1.65 (2.00—1.35) | 38 (66—12) | 126 (188—96) | 14 (17—11) | 233 (330—165) |
| **Prednisone in Small Intestine 1 mg after Ligation of Pylorus** | 10 | 78 | 24 | 1 | 4 | 2 | 5/10 | 32 | 0.33 (0.41—0.26— | 1.63 (2.00—1.30) | 40 (72—11) | 113 (130—82) | 15 (20—10) | 213 (270—165) |
| Control | 12 | 40 | 6 | 1 | 1 | 1 | 3/12 | 10 | 0.36 (0.45—0.27) | 1.50 (2.00—1.30) | 51 (75—26) | 125 (194—83) | 16 (20—12) | 186 (234—120) |

Microscopic examination showed that in the majority of the cases the applied tabloid piece or suspension was in the submucosa of the stomach wall and only in a couple of cases in the muscularlayer or under the serosa. In 3 cases erosions of the mucous membrane were found, in 5 ulcers that reached the submucosa and in 6 perforations.

The results shown in table 1 (some typical examples in plates 1—4), we feel, speak strongly in favour of the view that in tests of this type ulceration is mainly due to mechanical irritation caused by the treatment. Ulceration was an almost regular phenomenon when a hydrocortisone or placebo tabloid piece was applied into the stomach wall. If, on the other hand, hydrocortisone or prednisone was injected as a suspension into the same site, ulcers appeared only exceptionally. On the basis of this investigation, it is obvious that hydrocortisone and prednisone scarcely have any direct ulcer-producing effect upon the stomach wall. The table also shows that inflammation in the stomach wall produced by the administration of corticosteroids had scarcely such effect either. The local application of a hydrocortisone tabloid, hydrocortisone acetate or prednisone suspension into the stomach wall can scarcely have any systemic effect because, when administered in this way, they are almost insolube and absorb slowly from the tissue.

The results concerning the effect of prednisone upon Shay rats illustrated in table 2, we think, speak in favour of the trend seen from table 1. Prednisone given straight into the pyloric ligated stomach by means of a catheter did not cause any ulceration that differed from that of the controls, while prednisone administered into the small intestine clearly raised the ulceration rate. The rise was not due to an appearance of ulcers in a greater number of individuals but to the fact that those who had ulcers showed in average three times more of them. In other words, in animals with a disposition to ulcearation prednisone was apt to increase this disposition if given in such a way that it could be absorbed and have a systemic effect. From the bag formed by the pyloric ligated stomach constantly swelling with secretion gathering in it, prednisone has hardly any chance to become absorbed and its systemic effect can scarcely appear. Locally, however, it probably affects the glandular

epithelium of the mucous membrane, but it did not increase ulceration. Not one of the figures which in table 2 show the the secretion of the stomach after pyloric ligation differs to any notable extent from corresponding figures in other groups. In the secretion conditions there did not appear any remarkable differences either between those animals which had ulcers and those which showed no ulceration. The rise of the ulceration rate in pyloric ligated rats caused by systemic prednisone effect is thus difficult to ascribe to changes in the secretion.

On the basis of our tests it might be said that a prednisone or hydrocortisone application which takes place straight into the mucous membrane of the stomach or into the stomach wall has no ulceration-increasing effect, while it is possible to increase ulceration in pyloric ligated rats by means of systemic prednisone effect in such a way that there will be more ulcers in certain individuals, but not in such a way that there would be an increase in the number of individuals suffering from ulceration.

## SUMMARY

The writers have applied a piece of a hydrocortisone or placebo tabloid, hydrocortisone acetate suspension and prednisone suspension into the stomach wall between the serosa and muscularis propria and given pyloric-ligated rats prednisone by means of a catheter straight into the stomach, or injected it into the small intestine. They came to the following conclusions:

1) The mechanic irritation caused by the application of a piece of a hydrocortisone or placebo tablet between the serosa and muscularis propria almost regularly leads to ulceration of the gastric mucosa. This did not happen if the same doese of hydrocortisone or prednisone was applied in a less mechanically traumatizing way into the same spot in the form of a suspension. In this case ulceration was rare, whether the procedure caused inflammation or not.

2) The administration of prednisone straight into the stomachs of pyloric-ligated rats did not increase ulceration, while — on the other hand — prednisone given straight into the small intestine during similar conditions increased ulcer formation in

the individuals to about three-fold, although it did not increase the number of individuals suffering from ulceration. The administration of prednisone to pyloric ligated rats did not show any clear changes in the secretion conditions of their stomachs.

3) The results suggest that locally-applied glucocorticoids do not have any properties that directly cause ulcers in the mucous membrane or the wall of the stomach, but that in the conditions prevailing during these tests it is possible to increase individual ulceration by their systemic effect without any clear changes in the secretion conditions as regards the quantity, acidity or peptic activity of the gastric juice.

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## EFFECTS OF THE FINNISH SAUNA BATH ON THE ELECTROLYTE EXCRETIONS AND THE RENAL CLEARANCES

BY
**ERKKI HAAPANEN**

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